**INTRODUCTION**

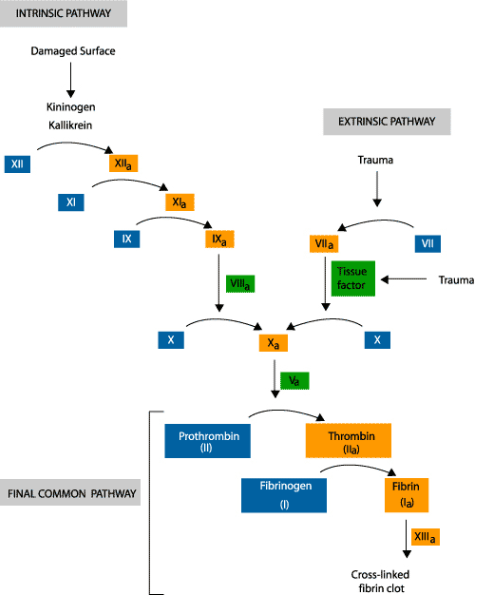
* 2 elements important for the formation of a thrombus
  + platelet aggregation
  + generation of a fibrin network (coagulation)’
* White thrombus
  + Mostly platelets
  + More form on arterial side of CV system
  + Can block blood flow to vital organs like the heart or brain and induce secondary thrombosis producing and MI or stroke
  + Antiplatelet drugs are used to prevent these
* Red Thrombus
  + Mostly fibrin and red cells
  + Tend to form more on the venous side of CV system
    - In the deep veins where blood flow is slower
  + These often form after surgery (esp. in the abdomen, pelvic organs, and hip) or when ppl are sedentary
  + DANGER—emboli breaking off and traveling to pulmonary artery🡪 PE
  + Anticoagulants and fibrinolytics are effective against these

**PLATELET AGGREGATION**

* Formation of a platelet plug
  + Platelet binding
    - Rupture of endothelium exposes collagen
    - Blood platelets bind collagen via Gp Ia/IIa complex🡪 platelet activation
    - Platelet binding is reinforced by further interaction mediated by vWF via platelet receptor GpIb/IX/V (this is important when blood flow is fast)
      * vWF (made in endothelium) has 2 functions
        + platelet adhesion
        + transport FVIII (made in liver) in blood stream and prolong it’s t½ life
        + \*vWD is most common inherited bleeding disorder
  + Platelet activation
    - Interaction with collagen activates platelets and induces release reaction secondary to elevated cytosolic Ca and activation of protein kinases
    - PLC🡪 ↑ IP3/Ca 🡪 Ca activates PLA2 🡪 release of arachidonic acid from membrane lipids🡪 synthesis of TXA2 (requires COX-1)
    - ADP, 5-HT, Platelet factor 4 (PF4) and fibrinogen are released from granules into the plasma
    - Platelets change shape🡪 activation of fibrinogen receptors (GpIIb-IIIa)🡪 insertion of negatively charged phospholipids in the outer leaflet of the membrane
    - TXA2 has 2 effects
      * Binds to receptors on VSM🡪 local vasoconstriction 🡪 ↓ blood loss
      * Amplifies process by activating other platelets that aren’t interacting w/ collagen
    - ADP activates platelets via P2Y12ADP receptor🡪 also activate IP3/Ca pathway
    - Thrombin can also activate platelets (most important platelet activator)
    - PF4 binds to glycosylated proteins in endothelial membranes and blocks the action of heparin-like natural anticoagulants
  + Platelet Aggregation
    - Fibrinogen can bind to its receptors on adjacent platelets and forms interplatelet bridges🡪 platelet aggregate🡪 platelet plug🡪 can prevent blood loss in a few minutes
    - vWF can bind to the same receptors and contributes to aggregation
    - **Bleeding Time Test**—time it takes for bleeding to stop after making a small shallow incision (Normal= 3—9.5 min)
  + Diseases caused by platelet Defects
    - Autosomal inherited (all ↑ bleeding)
      * vWD—very common
        + Autosomal Dominant (AD)
        + Defect/deficiency of vWF
      * Bernard-Soulier Dz—Autosomal Recessive (AR)
        + Defect in vWF receptor (GpIb/IX/V)
      * Glanzmann’s Thrombasthenia—AR
        + Defect in platelet fibrinogen receptor (GpIIb-IIIa)
      * Hemophilia A
        + Defect in VIIIa is most common cause
  + Desmopressin (DDAVP)
    - Arg-Vasopressin analog
      * Vasopressin (ADH) promotes water reabsorption in kidney’s
    - Given by oral tablets or IV infusion
    - Selective for V2 Receptors (cAMP)
    - The D-amino acid give it a longer t½ so don’t have to give the drug as frequently
    - Releases stores of vWF into blood from “Weibel-Palade bodies” in endothelial cells
    - ↑ vWF 🡪 ↑ in FVIII
    - used to tx bleeding episodes, and given prior to surgery for prophylaxis of bleeding in patients w/ type I vWD or mild hemophilia A
    - NOT FOR CHRONIC USE b/c vWF stores can become depleted.
* Inhibition of platelet Aggregation
  + Physiologic inhibition
    - PGI3 and NO released by endothelial cells inhibit the release rxn via ↑ cAMP and cGMP respectively
      * These two also dilate blood vessels so ↑ BF
    - ADP 🡪 AMP + adenosine + Pi by ecto-ADPase on endothelial cells
    - Thrombin interacts at receptors on endothelial cells to stimulate PGI2 and NO production
    - \*\*Drugs that ↑ NO or ↑ platelet cAMP are called “antiplatelet drugs”
  + Pharmacological inhibition
    - Aspirin blocks TXA2 synthesis
    - Ticlopidine and clopidogrel block ADP receptor
    - Abciximab, eptifibatide and tirofiban block fibrinogen receptor
    - Heparin and LMW Heparins indirectly inhibit thrombin
    - Epoprostenol (PGI2) and adenosine ↑ platelet and VSM cAMP 🡪 ↑ BF and inhibiting platelet aggr.
    - Dipyridamole, cilostazol block breakdown of cAMP by PDE’s
    - NO promotes vasodilation and inhibits platelet aggr.

**COAGULATION AND ANTICOAGULATION**

* General Principle
  + Coagulation is a process in which a fibrin clot is made
  + many of the steps involve trypsin-like serine proteases (clotting factors)
  + Coagulation cascade has a requirement of Ca and anionic phospholipids 🡪 help to localize coagulation to the site of injury
    - FII, FVII, FIX, FX posses 10-12 γ-carboxylated glutamate residues in the N-terminal region which provides binding sites for Ca—once bound to calcium then can bind the factors to negatively charged lipid from damaged endothelium or activated platelets
      * Glutamate 🡪 γ-carboxylated glutamate is a Vitamin K dependent carboxylation—so these factors are vitamin K dependent
* Coagulation
  + The “Extrinsic” Pathway



* + - Initiated by Tissue Factor (TF)=thromboplastin
      * Protein not normally found in the blood
      * TF is expressed in the membranes of SMC and fibroblasts and activated and exposed to blood following endothelial cell damage
    - TF Forms complex w/ VIIa
      * VII is activated by small amounts of VIIa, Xa, IXa, or XIIa already present in the blood stream
    - TF-FVIIa complex activates X & IX
      * IXa forms a fomplex w/ its cofactor VIIIa and activates X (important for continued production of Xa
      * Xa forms a complex w/ its cofactor—Va and activates prothrombin (factor II) producing thrombin
        + This activation releases thrombin from its γ-carboxyglutamated moiety so its free to migrate from the platelet membrane
      * Thrombin is the central enzyme in the coagulation process and has several functions:
        + Cleaves 2 small peptides (A and B) from fibrinogen 🡪 fibrin which polymerizes to form fibers
        + Activates platelets via platelet thrombin receptors on platelets
        + Promotes coagulation by activating Vii, V, and VIII
        + Promotes clot stabilization by activating XIII (w/o this will have bleeding disorder), which is a transamidase that crosslinks fibrin monomers and also cross links the protease inhibitor α2 antiplasmin to the network protecting it against digestion
  + Clotting Assay—prothrombin time (PT)
    - Extrinisic pathway is the basis for PT
      * TF, Ca, & negatively charged phospholipids are added to blood sample
    - PT determines INR which is used to monitor warfarin therapy and is always used as an assay for liver dysfunction
    - Doesn't require VIII or IX so if you have Hemophilia A or B this time wouldn’t be affected
  + The Intrinsic Pathway
    - Responsible for spontaneous clotting of blood in test tubes
    - Initiated by negatively charged surfaces (membranes of damaged endothelial cells and activated platelets, collagen and other subendothelial components, foreign surfaces like glass) which induce partial activation of XII. XIIa then activates prekallikrein 🡪 kallikrein
    - Kallikrein activates XII by proteolytic cleavage🡪XIIa
      * Kallikrein also release bradykinin by cleavage of HMW kininogen
      * Bradykinin induces pain, activates mast cells and has many of the same inflammatory properties as histamine
    - XIIa activates prekallikrein and XI🡪 XIa
    - Xia activates IX🡪 IXa
    - The rest is shared w/ extrinisic pathway in the common pathway
  + Clotting Assay—activated partial thromboplastin time (aPTT)
    - aPTT measures the intrinsic pathway
    - Kaolin (clay—used as a surface for factors to bind to), negatively charged phospholipids and Ca are added to the blood
    - Used to assay for hemophilia A (factor VIII) and B (factor IX) and to monitor patients being administered unfractionated heparin
* Hemophilias
  + Hemophilia A-inherited deficiencies of Factor VIII
  + Hemophilia B-inherited deficiencies of Factor IX
  + Hemophilia C-inherited deficiencies of Factor XI
  + Deficiencies of factor XII (Hageman factor), Prekallikrein and HMWK (high MW kininogen) are NOT associated w/ bleeding disorders
  + Summary of Coagulation Tests in Disorders

|  |  |  |  |
| --- | --- | --- | --- |
|  | Bleeding Time | PT (INR) | aPTT |
| Hemophilia A, B | Normal | Normal | **Long** |
| vWD | Long | Normal | Long (FVIII) |
| Heparin | Normal | (Long) | **Long** |
| Warfarin | Normal | **Long** | (Long) |

* Anticoagulation
  + Thrombomodulin
    - Thrombin also plays a central role in anticoagulation mechanisms (limits its own production)
      * Anticoagulant properties are turned on when it binds to thrombomodulin (expressed on normal endothelial cell membranes)
      * This gives it new properties
        + It is now inactivated more rapidly by antithrombin III (AT)

AT is a protesase inhibitor which inhibits thrombin, Xa, IXa, and Xia

Inhibition is accelerated when AT is bound to heparin sulfate groups on endothelial cell glycoproteins or heparin

* + - * + It is now unable to activate fibrinogen, FV or platelets
        + \*\*It is now able to activate Protein C (PC; a γ-carboxyglutamylated protease)

activated PC (APC) in conjunction w/ its cofactor—protein S, inactivates VIIIa and Va by proteolytic cleavage

\*\*source of some adverse effects of orgal anticoagulants—pts who are deficient in PC or have a FV mutation (factor V leiden—resistant to inactivation) are prone to thrombosis

* + TF Pathway Inhibitor (TFPI)
    - VIIa is inactivated by TFPI
    - TFPI first binds and inhbits Xa and then TFPI-Xa binds and inhibits TF-VIIa complex
    - This mechanism ensure that inhibition does not take place too soon
    - Since it terminates production of Xa, further propagation depends on the IXa-VIIIa complex explain the in vivo requirement for these factors for normal hemostasis
* Fibrinolysis
  + Fibrin is hydrolyzed by plasmin (P), a protease, to produce soluble peptides D, E, X, Y, and D-dimers (D-dimers indicate fibrin has been cross-linked🡪 indicates a clot)
  + Generation of plasmin
    - Generated from the inactive precursor plasminogen (Pg)
    - Pg bound to fibrin is activated by tissue plasminogen activator (tPA—activates fibrinolysis) which is released from endothelial cells and Pg🡪 Plasmin
    - Once plasmin is formed it can cleave Pg🡪 lys-Pg which has ↑ affinity for fibrin and is activated faster
    - Pg is also activated by urokinase type plasminogen activator (uPA)
      * Activates Pg bound to fibrin but does not bind to fibrin
  + Control of Fibrinolysis
    - Plasminogen activator inhibitor (PAI-1) is released from endothelial cells and prevents premature activatin of plasminogen
    - Fibrin is also protected against hydrolysis by α2-antiplasmin, which is cross-linked to fibrin by FXIIIa
  + Inactivation of Plasmin and tPA
    - Plasmin escaping into blood stream is able to digest fibrinogen, prothrombin, FVIII and V🡪 so inorder to protect plasma proteins plasmin and tPA are very rapidly inactivated by α2-antiplasmin (AP) and by α2-macroglobulin (MG)
    - tPA escaping into the blood is also blocked by circulating PAI-1
  + \*\*The breakdown of fibrinogen (unlike fibrin) does not yield D-dimers and normal levels of D dimers in the blood can help exclude a dx of thrombosis---high negative predictive value

